

CLAIMS

1 - Hybrid probe particles comprising a nanoparticle of gold of a diameter in the range extending from 2 to 30 nm onto the surface of which are grafted by gold-sulphur bonds, at least one, and preferably from one to 100, organic probe molecules on the one hand, and on the other hand, at least 10, and preferably 10 to 10000, molecules with luminescent activity.

2 - The hybrid probe particles as claimed in Claim 1, characterised in that the number of molecules with luminescent activity grafted on the surface of the nanoparticle of gold is at least 10 times greater than the number of grafted organic probe molecules.

3 - The hybrid probe particles as claimed in Claim 1 or 2, characterised in that 10 to 1000, preferably 100 to 500, molecules with luminescent activity are grafted onto the nanoparticle of gold.

4 - The hybrid probe particles as claimed in any one of Claims 1 to 3, characterised in that the molecules with luminescent activity are fluorescent organic dyes whereof the emission maximum deviates by at least 25 nm from the absorption maximum of the gold plasmon.

5 - The hybrid probe particles as claimed in any one of Claims 1 to 4, characterised in that the molecules with luminescent activity are electroluminescent or chemiluminescent compounds, for example derivatives of luminol.

6 - The hybrid probe particles as claimed in any one of Claims 1 to 4, characterised in that the molecules with luminescent activity are luminescent compounds whereof the wavelength of the light emitted is greater than the excitation wavelength, preferably at least 200 nm.

7 - The hybrid probe particles as claimed in any one of Claims 1 to 4, characterised in that the molecules with luminescent activity are lanthanide complexes.

8 - The hybrid probe particles as claimed in any one of Claims 1 to 4, characterised in that the molecules with luminescent activity are selected from among the derivatives of rhodamine and in particular those of lissamine rhodamine B.

9 - The hybrid probe particles as claimed in any one of Claims 1 to 8, characterised in that at most 75% of the nanoparticle of gold is covered by a cover material exhibiting dielectric characteristics allowing shift of the plasmon band of gold en outside the emission zone of the molecules with luminescent activity.

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10 - The hybrid probe particles as claimed in Claim 9, characterised in that the cover material is selected from among polysiloxanes, SiO_2 , ZrO_2 , Ln_2O_3 and lanthanide oxohydroxides.

10 **11 -** The hybrid probe particles as claimed in any one of Claims 1 to 8, characterised in that the molecules with luminescent activity are grafted to the nanoparticles of gold by means of a thiolated organic spacer, this spacer not being identical to the organic probe molecules.

15 **12 -** The hybrid probe particles as claimed in Claim 11, characterised in that the spacer contains from 6 to 50 carbon atoms and is for example selected from among mercaptophenols, dihydrolipoic acid and thio-poly(ethyleneglycol).

20 **13 -** The hybrid probe particles as claimed in any one of Claims 1 to 12, characterised in that the nanoparticle of gold has a diameter in the range extending from 4 to 20 nm, preferably in the range extending from 5 to 16 nm.

14 - The hybrid probe particles as claimed in any one of Claims 1 to 13, characterised in that 1 to 10 organic probe molecules are grafted onto the nanoparticle of gold.

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15 - The hybrid probe particles as claimed in any one of Claims 1 to 14, characterised in that the organic probe molecules are selected from among polynucleotides type DNA, RNA or oligonucleotides, proteins of antibody type, receptor, enzyme, enzyme/substrate complex, glycoproteins, polypeptides, glycolipides, oses, polyosides and
30 vitamins.

16 - The hybrid probe particles as claimed in Claim 14, characterised in that the organic probe molecules are thiolated oligonucleotides or attached to a thiolated spacer.

17 - The hybrid probe particles as claimed in any one of Claims 1 to 14, characterised in that the organic probe molecules are molecules allowing biotin - streptavidin interaction.

5 18 - The hybrid probe particles as claimed in any one of Claims 1 to 17, characterised in that 10 to 1000 other organic thiolated molecules, distinct from the organic probe molecules and the molecules with luminescent activity, are additionally grafted onto the nanoparticle of gold, these other organic thiolated molecules preferably comprising at least one alcohol, amine, sulphonate, carboxylic acid or phosphate function.

10 19 - A preparation process for hybrid probe particles as claimed in any one of Claims 1 to 18, characterised in that it comprises the following steps:

- preparing a colloidal suspension of nanoparticles of gold of a diameter in the range extending from 2 to 30 nm, by reduction of a gold salt, and in particular hydrogen tetrachloroaurate, in an aqueous phase or alcoholic and in the presence of citrate,
- 15 - adding to the resulting colloidal suspension an aqueous or alcoholic solution of thiolated organic probe molecules grafting onto the surface of the nanoparticles of gold by a gold-sulphur bond replacing citrate molecules,
- adding to the resulting colloidal suspension an aqueous or alcoholic solution of
- 20 molecules with luminescent activity grafting onto the surface of the nanoparticles of gold by a gold-sulphur bond replacing citrate molecules.

20 20 - A preparation process for hybrid probe particles characterised in that it comprises the following steps:

- preparing a colloidal suspension of nanoparticles of gold of a diameter in the range
- 25 extending from 2 to 30 nm, by reduction of hydrogen tetrachloroaurate, in an aqueous or alcoholic phase and in the presence of citrate,
- adding to the resulting colloidal suspension an aqueous or alcoholic solution of thiolated spacers functionalised with an ionisable function likely to react with the organic probe molecules or the molecules having luminescent activity to be grafted,
- 30 said spacers grafting onto the surface of the nanoparticles of gold by a gold-sulphur bond replacing citrate molecules,
- adding an aqueous or alcoholic solution of organic probe molecules functionalised to react with the ionisable function carried by the spacers grafted onto the surface of the nanoparticle of gold,

- and/or adding an aqueous or alcoholic solution of organic probe molecules functionalised to react with the ionisable function carried by the spacers grafted onto the surface of the nanoparticle of gold.

5 **21 -** A process for preparation of hybrid probe particles as claimed in Claim 19 or 20, characterised in that the reduction of the gold salt is done in the presence of tannic acid.

10 **22 -** The process for preparation of hybrid probe particles as claimed in any one of Claims 1 to 18, characterised in that it comprises the following steps:

- preparing a colloidal suspension of nanoparticles of gold of a diameter in the range extending from 2 to 30 nm, by reduction of gold salt, and in particular hydrogen tetrachloroaurate, in an aqueous or alcoholic phase and in the presence of NaBH_4 ,
- 15 - adding to the resulting colloidal suspension an aqueous or alcoholic solution of thiolated spacers functionalised with an ionisable function likely to react with the organic probe molecules or the molecules having luminescent activity to be grafted, said spacers grafting onto the surface of the nanoparticles of gold by a gold-sulphur bond,
- 20 - adding an aqueous or alcoholic solution of organic probe molecules functionalised to react with the ionisable function carried by the spacers grafted onto the surface of the nanoparticle of gold,
- adding an aqueous or alcoholic solution of organic probe molecules functionalised to react with the ionisable function carried by the spacers grafted onto the surface of the nanoparticle of gold.